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(57) Abstract

The present invention provides a nucleic acid molecule having a nucleotide sequence encoding human MOG, an autoantigen related to demyelinating autoimmune diseases. The present invention also provides recombinantly produced human MOG or antigenic fragments. Antigenic fragments of MOG, which can be prepared synthetically, represent T-cell epitopes of human MOG. Human MOG and fragments thereof are useful for diagnosing and treating autoimmune diseases. Further, methods of screening for, and developing therapeutics useful in the treatment of autoimmune diseases are also disclosed.

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USES OF MYELIN OLIGODENDROCYTE GLYCOPROTEIN AND PEPTIDE PORTIONS THEREOF IN PROTOCOLS RELATED TO AUTOIMMUNE DISEASE

Technical Field

The invention is directed to autoantigens and their relevant epitopes. More specifically, the invention concerns myelin oligodendrocyte glycoprotein (MOG) and the peptide regions thereof useful in diagnosis, treatment, and prevention of autoimmune conditions. Further, methods of screening for, and developing therapeutics useful in the treatment of, autoimmune disease are also disclosed.

Background Art

Autoimmune diseases are a significant human health problem and are relatively poorly understood. As there is no microbial or viral culprit apparently directly responsible, prevention, treatment and diagnosis of such diseases must be based on the etiology of the disease. This invariably involves a complex series of reactions of endogenous metabolic intermediates, structural components, cells, and so forth. Implicit, however, in the nature of an autoimmune condition is the notion that at least one autoantigen must be involved in creating the sequence of events that results in the symptoms. Autoimmune demyelinating diseases, such as multiple sclerosis, are no exception. Multiple sclerosis is an autoimmune disease in which T lymphocytes destroy central nervous system myelin. Multiple sclerosis is the most common cause of neurological disability associated with disease in Western countries.

A commonly used animal model for multiple sclerosis is experimental allergic encephalomyelitis (EAE) which can be induced, for example, in guinea pigs by administration of whole brain homogenate. F˙E, a demyelinating disease of the central nervous system, has been induced in mice with myelin basic protein (MBP) and has been accepted as a mouse model of human multiple sclerosis. It is well known in the art the EAE can be induced by immunization with MBP and complete Freund's adjuvant.

Animals so immunized exhibit symptoms of EAE, including, but not limited to, paralysis and often death. MBP is an autoantigen associated with human multiple sclerosis which continues to be studied. However, the search for other factors and other autoantigens which may contribute to this disease continues (*Immunology*, 2d Ed. J. Kuby,(1994) p. 451-457).

The apparent first indication of the autoantigen which came to be known as myelin oligodendrocyte glycoprotein (MOG) was disclosed in a paper by Lebar, R. *et al.* (*J Immunol* (1976) 116:1439-1446). This work reported the results of a study which identified an IgG2 antibody in the serum of EAE guinea pigs as responsible for the complement-dependent demyelinating activity of the serum. The relevant antibody reacted with the putative autoantigen present in the homogenate of central nervous system (CNS) myelin. This autoantigen was shown to be different from the encephalitogenic basic protein (BP) of CNS myelin.

In a subsequent paper, this unknown antigen, now designated M2, was identified as a surface antigen and detected in mouse, rabbit, rat, bovine and human CNS tissues as well as guinea pig. Further studies published as Lebar, R. *et al.*, *J Exp Immunol* (1986) 66:423-443, reported that M2 appeared as two bands at 27 and 54 kd, which bands represented glycoproteins. Monoclonal antibodies putatively specific for M2 were also reported in this paper.

In a 1987 paper (Linington, C. and Lassman, H. *J Neuroimmunol* (1987) 17:61-69) the role of M2, now called MOG, was partially elucidated. It was found that *in vivo* demyelinating activity of serum in rats was correlated with its anti-MOG antibody titer. The authors suggest that antibodies against MOG are involved in the pathogenesis of the model, in this case, chronic relapsing EAE. Further studies by this group reported a year later in Lassmann, H. *et al. Acta Neuropathol (Berl)* (1988) 75:566-576, further elucidated the relevant events in the EAE model. This study showed a relationship between anti-MOG antibody and high numbers of T-cells in determining the nature of the observed symptoms. In a still later paper by Kerleio deRosbo, N. *et al. J Neurochem* (1990) 55:583-587, it was shown that a monoclonal antibody directed against MOG induced demyelination in aggregating brain cell cultures. Piddelsden, S. *et al. Clin Exp Immunol* (1991) 83:245-250, further studied the importance of complement activation as significant in the course of EAE.

Sun, J. *et al. J Immunol* (1991) 146:1490-1495, directly studied MS in patients and evaluated both B- and T-cell response to MOG. This paper established that the T-cell response to MOG is Class II restricted and confirmed that MOG is an important antigen in multiple sclerosis.

Purified murine MOG has been shown to migrate as a 26-28 kd doublet band with a minor band at 53 kd on SDS-page immunoblots as reported by Matthieu, J.-M. and Amiguet, P. *Dev Neurosci* (1990) 12:293-302. Amiguet, P. *et al. J Neurochem* (1992) 58:1676-1682, had shown that chemical and enzymatic deglycosylation of murine MOG resulted in a single 25 kd peptide. Thus, murine MOG in its monomeric form is a 25 kd amino acid sequence. This article also disclosed the first approximately 26 amino acids at the N-terminus of the murine protein. Gardinier, M.V. *et al. J Neurosci Res* (1992) 33:177-187, isolated several rat MOG cDNAs and confirmed their identity by comparison with murine MOG N-terminal peptide sequence.

The rat cDNA obtained by Gardinier (*supra*) had an open reading frame that encoded a putative signal peptide of 27 amino acids followed by mature MOG peptide of 218 amino acids with a calculated molecular weight of 24,962 daltons. The N-terminal amino acid sequences obtained for the murine protein were similar to those deduced from the rat cDNA.

The present invention provides recombinant materials for the production of the human MOG protein as well as the complete amino acid sequence thereof. Using this information, the MOG protein or useful peptides representing portions of the amino acid sequence of MOG protein can be determined and are useful in the diagnosis and treatment of demyelinating autoimmune diseases in humans. Further, methods of screening for, and developing therapeutics compositions for, autoimmune disease is disclosed.

Disclosure of the Invention

The invention provides the complete amino acid sequence of human MOG protein (SEQ ID NO: 2) as well as recombinant materials for the production of the protein and fragments thereof. Knowledge of the amino acid sequence permits design of peptide portions thereof which are useful as well in diagnosis and treatment of autoimmune diseases.

Accordingly, in one aspect, the invention is directed to isolated and purified human MOG protein, optionally produced recombinantly, and to fragments thereof which modulate the course of development of symptomology in demyelinating autoimmune diseases. In another aspect, the invention is directed to recombinant materials and methods useful for the production of the MOG protein or peptide portions thereof. In still another aspect, the invention is directed to pharmaceutical compositions and methods of using them in mitigating the effects of demyelinating autoimmune .iseases.

In another aspect, the invention is directed to isolated and purified human MOG protein and peptides in which conservative substitutions have been made which exhibit characteristics of T-cell epitopes of the naturally occurring MOG protein and peptides.

In yet another aspect, the invention discloses a method for screening for demyelinating autoimmune disease and identifying therapeutic compositions comprised of MOG or fragments thereof which are capable diagnosing, preventing or treating multiple sclerosis in mammals, preferably, humans.

Brief Description of the Drawings

Figure 1 shows the complete nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO: 2) of DNA encoding human MOG protein.

Figure 2 shows peptides, designated by amino acid sequence, which are useful in the invention (SEQ ID NOS: 4-9, 11, 15, 16, and 42-72).

Figure 3 is a table showing the effect of each of the natural amino acids at certain positions on peptides binding to the MHC Class II product of DR4.

Figure 4 is a table showing a comparison of predicted and measured IC_{50} values for 12 peptides (SEQ ID NOS: 19-32) binding to the MHC Class II product of DR4.

Figure 5a shows peptides (20 mers) designated by amino acid sequence which correspond to SEQ ID NOS: 73-93.and are useful in the invention.

Figure 5b shows a peptide of human MOG 1-121,designated by amino acid sequence, which contains at least one T-cell epitope.

Figure 6 is a bar graph of data from Example 3 where the X-axis indicates wells containing 4 different cell lines, the 7 peptides tested are indicated by the different legends and the Y-axis indicates counts per minute where the data is expressed as 3H-thmidine incorporated (CPM) by each cell line in response to each individual peptide.

Modes of Carrying Out the Invention

The present invention provides nucleic acid sequences encoding human MOG, an autoantigen involved in demyelinating autoimmune diseases. It also provides peptides which represent subunits of the human MOG encoded by the nucleic acid molecules of the invention which are useful in moderating the autoimmune response. These subunits, which generally include on the order of at least 12-13 amino acids, are characterized by their correspondence to the T-cell epitope regions of the human MOG.

While the etiology of development of the symptoms of an autoimmune disease such as multiple sclerosis, is far from clear certain events are believed to be

critical to the progression of the condition. Demyelinating autoimmune diseases involve an attack by the immune system on the myelin sheath, resulting in what amounts to short circuits in the nervous system. The attack by the immune system is mediated by T-cells. T-cell responses to a particular antigen, in this case the human MOG autoantigen which is a part of myelin, require uptake and subsequent proteolytic cleavage of the antigen by antigen presenting cells and presentation of the antigen in the context of a Class II major histocompatibility complex (MHC) encoded protein which permits their recognition by the T-cells. Thus, the T-cell epitope regions of the autoantigen are those which are presented by the Class II MHC proteins to the T-cell receptors. The relevant T-cells can be rendered nonresponsive in an antigen-specific fashion in protocols by providing the T-cell epitope regions of the autoantigen, as is further described below.

One embodiment of the nucleic acid sequence encoding human MOG is shown in Fig. 1 (SEQ ID NO: 1), along with the deduced amino acid sequence (SEQ ID NO: 2). The encoded mature protein contains a 218 amino acids; the full length protein (including signal peptide) is 87% homologous with the rat MOG protein. As described above, the availability of the deduced amino acid sequence of human MOG provides the opportunity to design peptide fragments thereof which induce immune responses in mammals and peptide fragments which are T-cell epitopes - i.e., constitute those portions of the molecule which are recognized by T-cell receptors. These peptides and fragments are also included within the invention scope.

A host cell transformed with a vector (PVL1393, Pharmingen, CA) containing the nucleic acid sequence encoding human MOG was deposited with the American Type Culture Collection on September 8, 1994 and has accession number 75554.

However, the scope of the invention is not limited to the human MOG protein encoded by the amino acid sequence depicted in Figure 1, or to the specific nucleic acid sequence presented. Naturally occurring variants and deliberate mutations designed to modify the nucleic acid sequence *per se* or to modify the encoded protein are also included in the scope of the invention as further described below. With respect to naturally occurring variants, DNA sequence polymorphisms, especially those resulting in "silent" mutations which do not affect the amino acid sequence of the human MOG, but also sequence polymorphisms that do lead to changes in the amino acid sequence, are expected to exist in the human population. These variations in one or more nucleotides (up to about 1% of the nucleotides) of the sequence encoding MOG are a result of natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of the invention. Furthermore, there may be one or

more "family members" of MOG that are related in function and amino acid sequence to the MOG encoded by the DNA disclosed herein but encoded by separate genes. Such family members are also included within the definition of human MOG and the nucleotide sequences encoding it.

Isolated autoantigenic proteins or fragments thereof, that are novel and that are immunologically related to human MOG or fragments thereof, other than those already identified, are within the scope of the invention. These can be identified by antibody cross-reactivity or T-cell cross-reactivity. Such proteins or fragments thereof bind antibodies specific for the protein and peptides of the invention, or stimulate T-cells specific for the protein and peptides of this invention.

"Antigenic fragments" refer to an amino acid sequence having fewer amino acid residues than the entire protein and include fragments or peptides which induce an immune response in mammals, preferably humans, such as eliciting the production of IgG and IgM antibodies, or eliciting a T-cell response such as proliferation and/or lymphokine secretion and/or induction of T-cell anergy and/or modification of TH_1 and TH_2 subsets. Of particular interest are antigenic fragments that comprise T-cell epitopes. Peptides can be derived from the naturally occurring MOG sequence or peptides in which conservative amino acid substitutions have been made. Examples of each are shown in Figure 2 (SEQ ID NOS: 4-9, 11, 15, 16, and 42-72).

Preparation of Nucleic Acids

Nucleic acid molecules containing a sequence encoding human MOG or an antigenic fragment thereof may be obtained by reverse transcription mRNA present in human brain or other CNS tissue, as well as from genomic DNA, and can most conveniently be prepared using standard solid phase techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura *et al.* U.S. Patent No. 4,598,049; Caruthers *et al.* U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071, incorporated by reference herein). The nucleic acid molecules of the invention also include RNA which can be transcribed from the DNA prepared as above.

Preparation of Human MOG and its Fragments

The present invention also provides expression systems and host cells transformed with these systems for production of the encoded protein. Host cells include bacterial cells such as *E. coli*, insect cells (baculovirus), yeast, or mammalian cells such as

Chinese hamster ovary cells (CHO). Suitable host cells and expression vectors containing relevant promoters, enhancers and other expression control elements may be found in Goeddel, *Gene Expression Technology: Methods in Enzymology* **185**, Academic Press, San Diego, California (1990). Other suitable host cells and expression vectors are known to those skilled in the art.

Expression in eucaryotic cells such as mammalian, yeast, or insect cells can lead to partial or complete glycosylation and/or formation of relevant inter- or intra-chain disulfide bonds of recombinant protein. Examples of vectors for expression in yeast *S. cerevisae* include pYepSec1 (Baldari. *et al.*, (1987) *Embo J.* **6**:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* **30**:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* **54**:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith *et al.*, (1983) *Mol Cell Biol* **3**:2156-2165) and the pVL series (Lucklow, V.A., and Summers, M.D., (1989) *Virology* **170**:31-39). Generally COS cells (Gluzman, Y., (1981) *Cell* **23**:175-182) are used in conjunction with such vectors as pCDM 8 (Aruffo, A. and Seed, B., (1987) *Proc Natl Acad Sci USA* **84**:8573-8577) for transient amplification/expression in mammalian cells, while CHO (dhfr⁻ Chinese Hamster Ovary) cells are used with vectors such as pMT2PC (Kaufman *et al.* (1987), *EMBO J.* **6**:187-195) for stable amplification/expression in mammalian cells. Vector DNA can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, or electroporation. Suitable methods for transforming host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks.

Although those skilled in the art use various methods of expression, expression in procaryotes is most often carried out in *E. coli* with either fusion or non-fusion inducible expression vectors. Fusion vectors usually add a number of NH_2 terminal amino acids to the expressed target gene. These NH_2 terminal amino acids often are referred to as a reporter group. Such reporter groups usually serve two purposes: 1) to increase the solubility of the target recombinant protein; and 2) to aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the reporter group and the target recombinant protein to enable separation of the target recombinant protein from the reporter group subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5

(Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase, maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Inducible non-fusion expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). While target gene expression relies on host RNA polymerase transcription from the hybrid trp-lac fusion promoter in pTrc, expression of target genes inserted into pET 11d relies on transcription from the T7 gn10-lac 0 fusion promoter mediated by coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident g prophage harboring a T7 gn1 under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy would be to alter the coding sequence of the gene so that the individual codons for each amino acid would be those preferentially utilized in highly expressed *E. coli* proteins (Wada *et al.*, (1992) *Nuc Acids Res* 20:2111-2118). Such alteration of nucleic acid sequences of the invention could be carried out by standard DNA synthesis techniques.

Upon expression of the encoding gene, the recombinant protein or peptide product may be secreted and harvested from the medium. Alternatively, the protein may be retained cytoplasmically and the cells harvested, lysed and the protein isolated and purified. Suitable media for cell culture are well known in the art. The protein and peptides of the invention can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying proteins and peptides including ion-exchange chromatography, gel filtration chromatography, metal affinity chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with specific antibodies. The terms isolated and purified are used interchangeably herein and refer to peptides, protein, protein fragments, and nucleic acid molecules substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Accordingly, an isolated peptide is produced recombinantly or synthetically and is substantially free of cellular material and culture medium or substantially free of chemical precursors or other chemicals.

Antigenic Fragments and the "Antigenic" Response

Fragments of the invention protein that elicit a desired antigenic response (referred to interchangeably herein as antigenic fragments or peptides) may be obtained, for example, by screening peptides corresponding to portions of the protein. These peptides may be chemically synthesized using techniques known in the art, or produced recombinantly, or through proteolysis. For example, the protein may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or preferably divided into overlapping fragments of a desired length. The fragments are tested to determine their antigenicity (e.g., the ability of the fragment to induce an immune response in a mammal). If fragments of the protein are to be used for therapeutic purposes, then the fragments which are capable of eliciting a T-cell response, such as stimulation (i.e., proliferation or lymphokine secretion) and/or are capable of inducing T-cell anergy are particularly desirable.

The isolated protein or preferred antigenic fragments thereof, when administered to an individual subject to demyelinating autoimmune disease, are capable of modifying the B-cell response, T-cell response, or both the B-cell and the T-cell response of the individual to the autoantigen, or can be shown to result in a diminution of symptoms. As referred to herein, a diminution in symptoms includes any reduction in demyelination characteristic of the disease condition following a treatment regimen with a peptide or protein of the invention. This diminution in symptoms may be determined subjectively or clinically.

Human T-cell stimulating activity can be tested by culturing T-cells obtained from a subject having an autoimmune condition with the autoantigen and/or a peptide derived from the autoantigen and determining whether proliferation of T-cells occurs in response to the autoantigen and/or peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for responses by T-cells to peptides can be calculated as the maximum CPM in response to a peptide divided by the control CPM. A stimulation index (S.I.) equal to or greater than two times the background level is considered "positive". Positive results are used to calculate the mean stimulation index for each peptide for the group of patients tested. Preferred peptides of this invention comprise at least one T-cell epitope and have a mean T-cell stimulation index of greater than or equal to 1.5. A peptide having a mean T-cell stimulation index of greater than or equal to 1.5 in a significant number of patients tested (i.e. at least 10% of patients tested) is considered useful as a therapeutic agent. Preferred peptides have a mean T-cell stimulation index of at least 1.5, more preferably at least 2.0 to 3.0.

Preferred peptides can also be identified by their ability to effect a relatively higher frequency of T-cells in a patient. This frequency is measured by generating multiple identical cultures from one patient with limiting numbers of lymphocytes and the autoantigen and/or a peptide from the autoantigen. Individual cultures are analyzed for positive reactivity with a peptide, as defined by stimulation index (described above). The frequency of peptide-reactive T-cells is the percentage of cultures from the patient that show a positive stimulation index.

In addition, preferred peptides have a positivity index (P.I.) of at least about 100, more preferably at least about 200 and most preferably at least about 300. The positivity index for a peptide is determined by multiplying the mean T-cell stimulation index by the percent of individuals, in a population of autoimmune patients (e.g., preferably at least 15 individuals, more preferably at least 30 individuals or more), who have a T-cell stimulation index to such peptide of at least 1.5, more preferably at least 2.0. Thus, the positivity index represents both the strength of a T-cell response to a peptide (S.I.) and the frequency of a T-cell response to a peptide in a population of autoimmune individuals.

In order to determine precise T-cell epitopes by, for example, fine mapping techniques, a peptide having T-cell stimulating activity and thus comprising at least one T-cell epitope as determined by T-cell biology techniques is modified by addition or deletion of amino acid residues at either the amino or carboxy terminus of the peptide and tested to determine a change in T-cell reactivity to the modified peptide. If two or more peptides which share an area of overlap in the native protein sequence are found to have human T-cell stimulating activity, as determined by T-cell biology techniques, additional peptides can be produced comprising all or a portion of such peptides and these additional peptides can be tested by a similar procedure. Following this technique, peptides are selected and produced recombinantly or synthetically. Peptides are selected based on various factors, including the strength of the T-cell response to the peptide (e.g., stimulation index) and the frequency of the T-cell response to the peptide in a population of autoimmune subjects. The physical and chemical properties of these selected peptides (e.g., solubility, stability) are examined to determine whether the peptides are suitable for use in therapeutic compositions or whether the peptides require modification as described herein. The ability of the selected peptides or selected modified peptides to stimulate human T-cells (e.g., induce proliferation, lymphokine secretion) is determined.

A T-cell epitope-containing peptide of the invention, when administered to a subject in a therapeutic treatment regimen is capable of modifying the response of the individual to the autoantigen.

Preferred peptides of the invention comprise at least one T-cell epitope of the full length protein and accordingly the peptide comprises at least approximately 7, preferably at least about 12-40, and more preferably 13-30 amino acid residues. The peptides may contain tandem repeats of a single epitope and/or more than one epitope. For purposes of therapeutic effectiveness, preferred therapeutic compositions of the invention preferably comprise at least two T-cell epitopes. Additionally, therapeutic compositions comprising one or more preferred isolated peptides of the invention preferably comprise a sufficient percentage of the T-cell epitopes of the entire protein such that a therapeutic regimen of administration of the composition results in amelioration of disease symptoms. Synthetically produced peptides of the invention comprising less than approximately forty-five amino acid residues, and most preferably less than approximately thirty amino acid residues are particularly desirable as increases in length may result in difficulty in peptide synthesis. Peptides of the invention may also be produced recombinantly as described above, and it is preferable that peptides of 45 amino acids or longer be produced recombinantly.

Isolated antigenic peptide fragments which have T-cell stimulating activity, and thus comprise at least one T-cell epitope are particularly desirable. In referring to an epitope, the epitope will be the basic element, or smallest unit of recognition by a receptor, particularly immunoglobulins, histocompatibility antigens, and T-cell receptors, where the epitope comprises amino acids of the native protein. Amino acid sequences which mimic those of the epitopes can also be used. A T-cell epitope is the basic element, or smallest unit of recognition by a T-cell receptor, where the epitope comprises amino acids in the autoantigen essential to receptor recognition. Amino acid sequences which mimic those of the native T-cell epitopes are also within the scope of this invention. T-cell epitopes are believed to be involved in initiation and perpetuation of the autoimmune response. These T-cell epitopes are thought to trigger early events at the level of the T helper cell by being presented by an appropriate HLA molecule on the surface of an antigen presenting cell, thereby stimulating the T-cell subpopulation with the relevant T-cell receptor for the epitope. These events lead to T-cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site of antigen/T-cell interaction, and activation of the B-cell cascade leading to the production of antibodies.

Exposure of a subject to a peptide or protein which comprises at least one T-cell epitope of the autoantigen may tolerize, anergize or otherwise modify appropriate T-cell subpopulations such that they become non-responsive to the autoantigen and do not participate in stimulating an immune response. In addition, administration of a protein or peptide which comprises at least one T-cell epitope, may modify the lymphokine secretion profile as compared with exposure to the naturally-occurring autoantigen (e.g., result in a decrease of IL-4 and/or an increase in IL-2 causing a modification of TH_1 and TH_2 populations). Furthermore, exposure to such a protein or peptide may influence T-cell subpopulations which normally participate in the response to the autoantigen such that these T-cells are drawn away from the site(s) of normal exposure to the autoantigen (e.g., tissues of the CNS) to the site(s) of therapeutic administration of the protein or peptide derived therefrom. This redistribution of T-cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the autoantigen, resulting in a diminution in symptoms.

In determining which peptides are T-cell epitope-containing peptides for a specific disease, those skilled in the art might approach the task in many ways each of which is acceptable. In the instant invention, the procedures selected are not meant to be limiting. The selection of one mode of approaching a task is not intended to exclude other modes which can accomplish the same end by alternative means by those skilled in the art, for example, the selection of likely T-cell epitope-containing peptide from a group of peptides. Generally, in the instant application, as further explained in more detail herein, a protein is purified and analyzed, peptides are selected for testing, the selected peptides are produced, and the selected peptides are tested for properties characteristic of T-cell epitopes.

T-Cell Epitope Regions of Human MOG

Peptides derived from human MOG which moderate the response of a subject to the MOG autoantigen are also included in the invention. Likely candidates for such peptides can be tested for the effect on T-cell proliferation, as previously discussed and/or testing for a candidate's affinity for binding HLA DR proteins (procedures for obtaining HLA DR proteins is discussed in detail in the section below entitled "Purification and Analysis of HLA DR proteins") . Such peptides can be identified, for example, by examining the structure and selecting appropriate regions to be produced as pe[illegible]s (via recombinant expression systems, synthetically or otherwise) to [illegible] examined

for ability to influence B-cell and/or T-cell responses, and selecting peptides containing epitopes recognized by these cells. One method of identifying such peptides includes dividing the human MOG protein antigen into non-overlapping, or overlapping peptides of desired lengths and synthesizing, purifying and testing those peptides to determine whether the peptides comprise at least one T cell epitope using any number of assays. In another method, an algorithm is used for predicting those peptides. Other methods known to those skilled in the art may also be employed. In the instant application, certain of the peptides were selected using the algorithm as discussed in more detail herein.

Thirty-seven (37) naturally occurring 13-mers (together with three analogs of the naturally occurring peptides) are shown in Firgure 2 (SEQ ID NOS: 4-9, 11, 15, 16, and 42-72). ; the amino acid length is arbitrarily chosen. These peptides were chosen according to an algorithm that predicts optimal MHC class II binding. In order for the peptide to be able to bind an appropriate T-cell receptor, it is necessary that it be able to bind class II MHC proteins. It is possible to remove 1-2 amino acids from the N- and/or C-terminus of each and still retain MHC binding activity. These peptides are designated by amino acid sequence and represent the indicated regions of the mature amino acid sequence shown as positions 1-218 of Figure 1 (SEQ ID NO: 2). These subunit peptides are characterized by their ability to bind Class II MHC proteins so as to have the ability to be presented effectively as T-cell epitopes.(Rothbard, et al., *The EMBO Journal* 7: 93-100, 1988). It has been found that a necessary (but not necessarily sufficient) condition for binding to Class II MHC proteins is the presence of a hydrophobic side chain residue, preferably a tyrosine, phenylalanine or tryptophan residue, and less desirably isoleucine, leucine, valine or methionine residue spaced at a 4-amino acid distance from a small amino acid residue such as glycine, alanine, serine, threonine or cysteine. All of the MOG peptides shown in Figure 2 (SEQ ID NOS: 4-9, 11, 15, 16, and 42-72) fulfill these minimum conditions. As further shown in Figure 2, all of these peptides have been tested with respect to their ability to bind MHC proteins encoded by certain alleles. As shown by the IC_{50} values in the figure, most of these peptides bind tightly to both DRB1*0101 and DR B1*1501. The specifics of peptide binding are contained in the section on <u>Inhibition assays</u> described below. Figure 2 is intended to be a representative example of the algorithm for selecting and is quite useful. It is, however, not all inclusive as other possible useful antigenic peptides may exist.

The requirement for the 4 amino acid spacing between the hydrophobic residue and small residue used as the basis for identifying the peptides as described in the preceding paragraph has been verified using experimental protocols as follows: The design of the experiments assumed that all peptides bind in approximately identical

locations, oriented by interactions with the peptide backbone and adopt closely related conformations. The design of the experiments also assumed that the binding site of MHC Class II molecules can be divided into separate subsites that differentially contribute to binding, that the overall free energy of binding is the sum of the advantageous and deleterious contacts a peptide makes with the pockets, and that the interaction between the peptide in each of the pockets can be viewed independently. The results of the experiments support these postulates, as well as a model of free energy of binding as a simple polynomial with separate terms for backbone interactions in the side chains. For peptides of common length, the backbone terms would be constant while the contribution of side chains would vary depending on their structure and the chemical composition and size of the complementary pocket in the binding site. The relative importance of each side chain position will vary depending on the allele and the isotype, but in general peptide binding can be viewed as the sum of separate independent events.

Thus, the results of applicants' experiments show that the apparent affinity of any sequence of common length can be predicted within a factor of 2 or 3 of the experimentally determined values based on a database of the relative effects of the natural amino acids in each position of model peptides. All possible monosubstituted analogs at the central 11 positions of the simplified peptide, AAYAAAKAAAAAA (SEQ ID NO: 33) were synthesized and assayed for binding to DRB1*0401 (formerly called DR4DW4). From the measured IC_{50} values of each analog, a ratio relative to the parent simplified peptide was calculated. These ratios and the measured values are shown in Figure 3. The ratios were then used to predict the affinity of 12 unrelated natural sequences corresponding to peptides known to bind DRB1*0401 with a wide range of affinity as shown in Figure 4 (SEQ ID NOS: 19-32). The data shown on Figures 3 and 4 verify that the model is useful in predicting the 4 amino acid spacing and is consistent with experimental results. Using this model, candidates of 13mers were selected as likely T-cell epitope-containing peptides.

Purification and Analysis of HLA DR proteins

The procedures used for detergent solubilization and affinity purification of HLA-DR (human MHC protein) molecules were similar to those described by Gorga (*J.Biol.Chem.* 262: 16087-16094, 1987) and Buelow (*Eur.J.Immunol.*23:69-76, 1993). Briefly, CHO cells transfected with the genes for the DRB1*0101 and DRB1*1501.alleles (Marshall et al., *J.Immunol.*, 152:4946-4957, 1994) were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS), 2mM glutamine, and anitbiotics. Cells were harvested, washed in PBS< lysed with 1% NP-40 and the supernatant separated from

nuclear debris by centrifugation. The solubilized MHC class II proteins were affinity purified using the monoclonal antibody LB3.1, coupled to Sepharose CL4B. Class II proteins were eluted with 1% octyl-β-D-glucopyranoside (octyl glucoside), 50mM phosphate pH 11.5 and immediately neutralized using 1M phosphate pH 6.0. Purified αβ heterodimers were isolated by size exclusion using a 60 cm x 2.5 cm diameter column of Bio-Gel A0.5. The fractions containing the heterodimers were concentrated using Amicon Centri-Prep® 30 devices to a nominal concentration of 500 μg/ml.

The purity of the material was assayed by SDS-PAGE, high performance size exclusion chromatography (HPSEC), and Edman sequencing as previously described (Buelow et al., 1993). The HPSEC column was a BIOSEP SEC-S3000 (300 x 7.5 mm) (Phenomenex), eluted using a buffer system of PBS containing 1.0% octyl glucoside and 1.0% acetonitrile at a flow rate of 0.800 ml/min (approximately 25 minutes per run). The fluorescence detector was set to monitor tryptophan fluorescence (λ_{ex} = 282 nm, λ_{em} = 348 nm).

Inhibition assays

Peptide binding assays were performed as previously described (Hill et al.,1994). Briefly, affinity purified class II proteins (10nM) were incubated with serial dilutions of the test peptide and a fixed concentration of biotinylated HA 307-319 (2 nM) in PBS containing 1.0% octyl glucoside at pH 6.5 in 96 well polypropylene plates (Costar) for 16 hours at 37°C. The DR-peptide complexes (50μl) were transferred, in duplicate, to wells of a 96-well microtiter plate precoated with the monoclonal antibody LB3.1 and blocked with fetal calf serum. Excess peptide was removed by washing with PBS containing 0.02% Tween 20 and 0.05%NaN_3. Europium labeled streptavidin (Pharmacia) was added and incubated overnight. After washing, a solution of 0.1M acetate/phthalatebuffer, pH 3.2, containing 0.1% Triton X-100, 15 μM 2-naphthoyltrifluoroacetone and 50 μM tri-N-octylphosphine oxide was added to release the chelated europium from strepavidin. The resulting fluorescence (which was proportional to the amount of bound biotinylated HA 307-319) was measured using a fluorescent plate reader (DELPHIA LKB/Pharmacia). The data were analyzed by fitting the data to a binding function that calculated the concentration of test peptide to bind to a specific class II protein can be ranked by the corresponding IC_{50} values, with the lower values corresponding to better binding peptides.

T-cell assay used to identify MOG epitopes

T-cell assays were performed to further refine the identification of T-cell epitopes. Peripheral blood lymphocytes were isolated from the blood of a human volunteer HLA-DR2 positive donor using the techniques outlined in Example 2 herein. The MOG peptides selected for testing were:

MOG 1-13	GQFRVIGPRHPIR (SEQ ID NO: 42)
MOG 20-32	VELPCRISPGKNA (SEQ ID NO: 5)
MOG 70-82, A78	ELLKDAIGAGKVT (analog to MOG 70-82) (SEQ ID NO: 8)
MOG 88-100,K89,S98	VKFSDEGGFTSFF (analog to MOG 88-100) (SEQ ID NO: 9)
MOG 103-115	HSYQEEAAMELKV (SEQ ID NO: 55)
MOG 118-130	PFYWVSPGVLVLL (SEQ ID NO: 56)
MOG 170-182	PHFLRVPCWKITL (SEQ ID NO: 15)

Results of one such study of T-cell response to human MOG peptides is illustrated in Figure 6. The results have been interpreted and indicate that MOG 1-13 (SEQ ID NO: 42) is a borderline T-cell epitope-containing peptide. Furthermore, the same data strongly indicates that MOG 103-115 (SEQ ID NO: 55) contains at least one epitope. Still further, these results do not preclude the possibility that less than the entire 13 mer peptide could be a T-cell epitope-containing peptide.

Further, T-cell response of a human patient suffering from Multiple Sclerosis is discussed in Example 4, where a mixture of MOG peptides (20 amino acids in length and shown in Figure 5a) was found to contain at least one T-cell epitope for the auto antigen responsible for Multiple Sclerosis. Furthermore, these results do not preclude the possibility that less than an entire 20 mer peptide could be a T-cell epitope-containing peptide.

The structure of the protein or peptides of the invention can be modified for such purposes as increasing solubility, enhancing therapeutic or prophylactic efficacy, or stability (e.g., shelf life *ex vivo* and resistance to proteolytic degradation *in vivo*), or generally by conservative substitutions and modifications. A modified protein or peptide can be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion, or addition, to modify immunogenicity, or to which a component has been added for the same purpose. Figure 2 shows 3 such amino acids, Human MOG 70-82,A78 (SEQ ID NO: 8); Human MOG 74-86,A78 (SEQ ID NO: 53); and Human MOG 88-100, K89, S98 (SEQ ID NO: 9).

Modification must be made in such a way that the ability to recognize the appropriate T-cell subset is not altered. It is generally understood in the art which

locations of amino acid side chains are oriented toward the upper surface of the APC and which point inward. Those that point inward are suitable candidates for modification since they clearly are less likely to affect binding to the appropriate T-cell receptor.

One embodiment of the present invention features a peptide which comprises at least one T-cell epitope of the protein and includes the regions of the peptides as shown in Figures 2, 5a and/or 5b that are significant for T-cell receptor binding or the modified forms thereof as above described, optionally extended at the N- and/or C-terminus with irrelevant amino acid sequence.

Another embodiment of the present invention provides peptides comprising at least two T-cell epitopes as described above. The T-cell epitopes may be identical or may be different T-cell epitopes appropriate for human MOG. As further described above, the T-cell epitopes are typically at least 7 amino acids, preferably 12-40 amino acids, even more preferably 13-30 amino acids in length. If desired, the amino acid sequences of the T-cell epitopes can be joined by a linker to increase sensitivity to processing by antigen-presenting cells. Such linker can be any non-epitope amino acid sequence or other appropriate linking or joining agent. In preferred peptides comprising at least two T-cell epitopes, the epitopes are arranged in the same or a different configuration from a naturally-occurring configuration of the epitopes in the native human MOG protein. For example, the T-cell epitope(s) can be arranged in a contiguous or noncontiguous configuration. Noncontiguous is defined as an arrangement of T-cell epitope(s) which contains additional residues between the epitopes. Furthermore, the T-cell epitopes can be arranged in a nonsequential order (e.g., in an order different from the order of the amino acids of the native protein from which T-cell epitope(s) are derived). A peptide of the invention can comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 60% or more of the T-cell epitopes of human MOG.

Preferred peptides comprise various combinations of two or more of the above-discussed T-cell epitopes. Preferred peptides comprising a combination of two or more epitopes are those wherein the peptides include sequences selected from those in Figure 2 (SEQ ID NOS: 4-9, 11, 15, 16, and 42-72), Figure 5a and Figure 5b.

A protein or peptide of the invention can be modified so that it maintains the ability to induce T-cell unresponsiveness and bind MHC proteins without the ability to induce a strong or any proliferative response when administered in immunogenic form. In this instance, critical binding residues for T-cell receptor function can be determined using known techniques (e.g., substitution of each residue and determination of the presence or absence of T-cell reactivity). Those residues shown to be essential to interact with the T-cell receptor can be modified by replacing the essential amino acid with

another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance or diminish, but not eliminate T-cell reactivity. In addition, those amino acid residues which are not essential for T-cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance or diminish T-cell reactivity, but does not eliminate binding to relevant MHC.

Additionally, peptides of the invention can be modified by replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance or diminish, but not to eliminate T-cell activity. It is believed that peptides that bind MHC with higher affinity should render T-cells immunopassive *in vivo* at lower concentrations. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which are present on the bound peptide can be modified by being replaced by another amino acid whose incorporation may enhance or diminish, but not eliminate T-cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid, or a methyl amino acid.

Another example of modification of proteins or peptides is substitution of cysteine residues preferably with alanine, serine, threonine, leucine or glutamic acid residues to minimize dimerization via disulfide linkages. In addition, amino acid side chains of peptides of the invention can be chemically modified. Another modification is cyclization of the peptide.

In order to enhance stability and/or reactivity, the protein or peptides of the invention can be modified to incorporate one or more polymorphisms in the amino acid sequence of the protein autoantigen resulting from any natural allelic variation. Additionally, D-amino acids, non-natural amino acids, or non-amino acid analogs can be substituted or added to produce a modified protein or peptide within the scope of this invention. Furthermore, proteins or peptides of the present invention can be modified using polyethylene glycol (PEG) according to the method of A. Sehon and co-workers (Wie *et al.*, *supra*) to produce a protein or peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a protein or peptide of the invention. Modification of proteins or peptides or portions thereof can also include reduction/alkylation (Tarr in: *Methods of Protein Microcharacterization*, J. E. Silver ed., Humana Press, Clifton NJ 155-194 (1986)); acylation (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds, *Selected Methods in Cellular Immunology*, WH Freeman, San Francisco, CA (1980), U.S. Patent 4,939,239; or mild formalin treatment (Marsh (1971), *Int Arch of Allergy and Appl Immunol* 41:199-215).

To facilitate purification and potentially increase solubility of protein or peptides of the invention, it is possible to add an amino acid reporter group to the peptide backbone. For example, hexahistidine can be added to a protein or peptide for purification by immobilized metal ion affinity chromatography (Hocbuli, E. *et al.*, (1988) *Bio/Technology* 6:1321-1325). In addition, to facilitate isolation of protein or peptides free of irrelevant sequences, specific endoprotease cleavage sites can be introduced between the sequences of the reporter group and the protein or peptide. In order to successfully desensitize an individual to a protein antigen, it may be necessary to increase the solubility of a protein or peptide by adding functional groups to the protein or peptide, or by omitting hydrophobic regions of the protein.

To potentially aid proper antigen processing of T-cell epitopes within a peptide, canonical protease sensitive sites can be engineered between regions, each comprising at least one T-cell epitope via recombinant or synthetic methods. For example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during recombinant construction of the peptide. The resulting peptide can be rendered sensitive to cleavage by cathepsin and/or other trypsin-like enzymes which would generate portions of the peptide containing one or more T-cell epitopes. In addition, such charged amino acid residues can result in an increase in the solubility of a peptide.

Site-directed mutagenesis of DNA encoding a peptide or protein of the invention can be used to modify the structure of the peptide or protein by methods known in the art. Such methods may, among others, include polymerase chain reaction (PCR) with oligonucleotide primers bearing one or more mutations (Ho *et al.*, (1989) *Gene* 77:51-59) or total synthesis of mutated genes (Hostomsky, Z. *et al.*, (1989) *Biochem Biophys Res Comm* 161:1056-1063). To enhance recombinant protein expression, the aforementioned methods can be applied to change the codons present in the cDNA sequence of the invention to those preferentially utilized by the host cell in which the recombinant protein is being expressed (Wada *et al.*, *supra*).

The isolated protein and/or antigenic fragments can be used in methods of diagnosing, treating, and preventing demyelinating autoimmune responses. Thus, the present invention provides therapeutic compositions comprising isolated human MOG (SEQ ID NOS: 1 and 2), or antigenic fragment thereof, and a pharmaceutically acceptable carrier or diluent. Furthermore, the isolated protein and/or fragments can be used in screening for the autoimmune disease and for developing candidates for therapeutic compositions.

Pharmaceutical Compositions

Administration of the therapeutic compositions of the present invention can be carried out using known procedures, at dosages and for periods of time effective to ameliorate the disease. In addition to compositions containing a single peptide or protein, mixtures of at least two peptides (e.g., a physical mixture of at least two peptides), each comprising at least one T-cell epitope of human MOG can also be provided such compositions can be administered in the form of a therapeutic composition with a pharmaceutically acceptable carrier of diluent.Still further, the therapeutic composition may contain peptides comprising at least two regions, each region comprising at least one T-cell epitope of MOG and which regions may be arranged in a configuration different from a naturally-occurring configuration of the regions in human MOG. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially.

Preferred compositions and preferred combinations of peptides which can be administered simultaneously or sequentially comprise peptides comprising amino acid sequences shown in Figures 2 (SEQ ID NOS: .4-9, 11, 15, 16, and 42-72), 5a (SEQ ID NOS. 73-80, 82,83) and 5b (the first 121 amino acids of human MOG protein SEQ ID NO. 2), more preferably, MOG 1-13 (SEQ ID NO. 42) and MOG103-115 (SEQ ID NO. 55).

Effective amounts of the therapeutic compositions will vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, and the ability of the protein or fragment thereof to elicit an antigenic response in the individual. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The protein, peptide or pharmaceutical composition thereof may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, sublingual administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, composition may include a coating with in a material to protect from the action of enzymes, acids and other natural conditions which may cause inactivation.

For administration by injection, for example, about 1μg-3mg and preferably from about 20μg-750μg of protein or peptide per dosage unit is typical. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions

(where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabenz, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (i.e., protein or peptide) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The protein or fragment thereof may be administered to an individual in an appropriate diluent or adjuvant, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Adjuvant is used in its broadest sense and includes any immune stimulating compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, (1984) *J. Neuroimmunol.* 7:27). For purposes of inducing T-cell nonresponsiveness, the therapeutic composition is

preferably administered in non-immunogenic form, e.g., one that does not contain adjuvant.

When protein or peptide is suitably protected, as described above, the protein or peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The protein and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations contain an effective amount. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 10 μg to about 200 mg of active compound.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieve, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

The present invention also provides compositions comprising at least two peptides (e.g., a physical mixture of at least two peptides), each comprising at least one T-cell epitope. Such compositions can be administered to an individual in the form of a therapeutic composition with a pharmaceutically acceptable carrier or diluent as

hereinbefore described. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially.

Diagnosis

The proteins or peptides of the present invention can be used in "purified" form for standardization of reagents for the diagnosis and treatment of autoimmune disease. The isolated and purified protein or peptide is also useful to prepare antisera or monoclonal antibodies for use in diagnosis. An animal such as a mouse or rabbit can be immunized with an immunogenic form of the isolated protein or isolated peptide, if necessary, conferring immunogenicity on a protein or peptide by coupling to carriers or by other techniques well known in the art. The protein or peptide can be administered in the presence of adjuvant, and progress of immunization can be monitored by detection of antibody titers in plasma or serum standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, antisera can be obtained and polyclonal antibodies isolated, if desired, from the serum. To produce monoclonal antibodies, antibody producing cells (lymphocytes) are harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Hybridoma cells can be screened immunochemically for production of antibodies reactive with the invention protein or peptide thereof. The antisera or monoclonal antibodies can be used to standardize reagents in standard assays.

Protein, peptides, or antibodies of the present invention can also be used for detecting and diagnosing autoimmune disease. For example, this could be done by combining blood, or blood products, obtained from an individual with an isolated antigenic peptide under conditions appropriate for binding of components in the blood (e.g., antibodies, HLA molecules, T-cells and B-cells) with the peptide(s) or protein, and determining the extent to which such binding occurs. Other diagnostic methods for autoimmune diseases which the protein, peptides or antibodies of the present invention can be used include paper radioimmunosorbent test (PRIST), enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA), and luminescence immunoassays (LIA).

Primers, Probes and Other Oligomers

The availability of the nucleotide sequence shown in Figure 1 (SEQ ID NO: 1) and of its complement (SEQ ID NO: 2) permit the design of various

oligonucleotides useful in therapeutic and diagnostic contexts. Modulation of the expression of the gene encoding MOG affects the progression of the autoimmune disease; in addition, progression can be monitored by monitoring expression using probes for RNA. Also, oligomers based on the nucleotide sequence disclosed in Figure 1 herein can be used in standard assay methods for detecting the MOG-encoding DNA or RNA.

By oligomers "based on" the sequence disclosed in Figure 1 (SEQ ID NO: 1) is meant oligomers that contain portions of this sequence, that are complementary to the sequence or portions thereof, that represent primers used to amplify portions of the sequence when large amounts of DNA are desirable (such as for genetic manipulation) as well as oligomers designed on the basis of the disclosed sequence which effect triple helix formation with the relevant portion of the duplex representing the MOG gene. Relevant design parameters for PCR primers, oligomers capable of hybridizing to single strand targets, and oligomers capable of triple helix formation with DNA duplexes are well known in the art. Thus, oligomers "based on" the DNA of Figure 1 (SEQ ID NO: 1) may have the same sequence as a portion of this DNA, the same sequence as the complement or portion thereof, or a different sequence but one which corresponds to that disclosed in Figure 1 (SEQ ID NO: 1) through art-known design parameters.

The oligomers having nucleotide sequences based on the nucleotide sequence shown in Figure 1 (SEQ ID NO: 1) may be conventional RNA or DNA polymers, or may be modified forms thereof as generally known in the art. For example, the phosphodiester bonds of the oligomers may be substituted by alternative linkages such as phosphorothioates, methylphosphonates and the like. In addition, alternative scaffolding for nucleotide bases has also been disclosed and such modifications are included within the scope of oligomers claimed herein.

The following examples are intended to illustrate, but not to limit, the invention.

Example 1

cDNA Encoding Human MOG Protein

In an initial attempt to obtain human DNA encoding MOG protein, a human cDNA library was subjected to the polymerase chain reaction (PCR) using 3' and 5' primers designed from the published rat MOG coding sequence of Gardinier *et al.* (*supra*). The human MOG sequence could not be obtained in this manner, putatively due to insufficient homology at the 5' and/or 3' ends of the human and rat sequences.

Therefore, four rat internal oligonucleotides were designed. Two of them were homologous to the top strand of the gene (primers 94-111 and 166-183 (SEQ ID NO: 34), base 1 starting at the ATG) and two were homologous to the bottom strand of the gene (primers 538-555 (SEQ ID NO: 35) and 685-702). The combination of primers 166-183 (SEQ ID NO: 34) and 538-555 (SEQ ID NO: 35) was successful in effecting the amplification of a fragment of the approximately 400 bp expected size from a human brain cDNA library. The sequence of these primers was:

a) 166-183: CAGAATCCGGGAAGAATGCCACGGGC (SEQ ID NO: 34); and

b) 538-555: CAGCGGCCGCACGGAGTTTTCCTCTCAG (SEQ ID NO: 35).

An EcoRI site is present in the 166-183 primer (SEQ ID NO: 34); a NotI site is present in the 538-555 primer (SEQ ID NO: 35).

The 400 bp PCR product was cloned into expression vector pVL1393 by digesting pVL1393 (Pharmingen CA) with EcoRI and NotI, digesting the amplified product with the same enzymes and ligating the resulting fragments. The insert was verified by digesting several clones derived from the ligated plasmids with EcoRI and NotI and sequencing the resulting 400 bp human MOG fragment. The resulting insert putatively lacks 184 bp of 5' sequence and 201 bp of 3' sequence, based on the 738 bp rat open reading frame.

Two primers were designed from the 400 bp insert from positions 346-363 top and bottom strands as follows:

5'-CAGAATTCTCAGGTTCTCAGATGAAGGA-3' (SEQ ID NO: 36); and

5'-AAGCGGCCGCTATCCTTCATCTGAGAACCT-3' (SEQ ID NO: 37).

wherein an EcoRI site is present in the first strand and a NotI site in the second. Underlined regions correspond to the MOG sequence.

The human MOG 346-363 top and bottom primers (SEQ ID NOS: 36 and 37) were used in combination with the above-mentioned 5' and 3' rat primers, respectively, to amplify the 5' and 3' missing ends of the gene from the same human brain cDNA library as previously used. A PCR product corresponding the 3' end of the gene was obtained, but the corresponding 5' end did not result.

The 3' fragment obtained had the expected 400 bp size and this fragment was cloned in pVL1393 and sequenced.

To obtain the 5' portion of the gene, a human brain medulla λgt10 library obtained from Clontech which had been previously amplified and had a titer of $8x10^{10}$

pfu/ml was screened following the protocol described by the manufacturer. The library was plated onto 12 large plates at 30,000 plaques/plate and the plaques were lifted onto nitrocellulose filters (2 replica filters/plate). Twelve filters lifted from the 12 different plates were then hybridized to a ^{32}P labelled probe corresponding to the human MOG internal 400 bp fragment initially cloned (positions 184-534). Twenty-two strong positives were obtained. A plug was picked for each positive from the original plates and incubated overnight with λ dilution buffer to elute the phage from the agar. The tube was then centrifuged and the supernatant transferred.

The DNA was amplified from each individual pool using either a λgt10 forward primer with an SstII site: 5'-CTTTTGAGCAAGTTCAGCCTGGTTAAG-3' (SEQ ID NO: 38) or a λgt10 reverse primer with an XhoI site: 5'-ACCTCGAGGAGGTGGCTTATGAGTATTTCTTCCAGGGTA-3' (SEQ ID NO: 39) as well as a human MOG internal primer top or bottom strand:

5'-GGTGCGGGAAAGGTGACTCTCA<u>GGATCC</u>GGAAT-3' (SEQ ID NO: 40)

or

5'-ATTCC<u>GGATCC</u>TGAGAGTCACCTTTCCCGCACC-3' (SEQ ID NO: 41).

The last two primers (SEQ ID NOS: 40 and 41) include a BamHI site (underlined in the sequences) naturally present in the human MOG sequence.

The primers were used in four different combinations: 1) forward top/internal MOG bottom; 2) reverse bottom/internal MOG bottom; 3) internal MOG top/reverse bottom; and 4) internal MOG top/forward top.

The first two combinations provided the 5' end of the gene (up to the BamHI site) and the last two, the 3' end of the gene. Both 5' and 3' portions include untranslated regions. Which of the two members of each combination actually resulted in the desired fragment depends on the orientation of the cDNAs cloned into λgt10.

The size of the fragments obtained varied from one pool to another. Five of the largest 5' fragments or 3' fragments were subcloned into the SstII and BamHI or BamHI and XhoI sites of the SK polylinker. Three clones from each pool were then sequenced to rule out the presence of PCR errors. This provided the complete sequence of the gene coding region as well as 174 bp of the 5' untranslated sequence.

The complete DNA sequence recovered (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) are shown in Figure 1.

The human MOG gene encodes a preprotein of 248 amino acids which has 87% homology with the 246 amino acids in the rat protein. The mature protein contains 218 amino acids, numbered 1-218 in Figure 1 (SEQ ID NO: 2). The mature protein begins at the glycine shown at position 1 and is derived fr ·the 248 amino acid

preprotein by cleavage from the presequence extending from the MET start codon to the alanine residue immediately preceding the glycine shown in position 1.

In addition, human MOG cDNA was cloned into a pET H6 vector for expression in E. coli. The pET.H6, obtained from Novagen (Madison, WI.), contains a sequence encoding six histidines which allows for purification of any recombinant protein over a Ni++ column. A truncated human MOG cDNA (no leader sequence and no transmembrane domains) encoding amino acids 1-121 of human MOG (the first 121 amino acids of SEQ ID NO. 2) was amplified by PCR using the following oligonucleotides:

5' primer:
5'-AGCTCGAGCCGCGGAGGGCAGTTCAGAGTGATA-3' (SEQ ID NO: 94)
3' primer:
5'-GACTCGAGTCACCAGTAGAAAGGATCTTC-3' (SEQ ID NO: 95)

The 363 bp PCR fragment was then cloned between the unique SstII and XhoI sites of pET.H6. After cloning, the cDNA was entirely sequenced.

Example 1A

Expression of truncated human MOG in SF-9 Insect cells and *E. coli*

SF-9 Expression

The PVL1393 transfer vector containing the truncated human MOG cDNA encoding amino acids 1-121 of human MOG (the first 121 amino acids of SEQ ID NO. 2) was cotransfected into SF-9 cells along with Baculogold linearized Baculovirus DNA (Pharmingen, San Diego, CA). The culture supernatant containing recombinant viruses was harvested after 4 days. The recombinant virus was plaque purified and subjected to 3 rounds of amplification to obtain a high titer viral stock. AF-9 cells were then infected with the viral stock at a MOI of 2.0. The supernatant from infected cells was harvested 48 hours after infection and applied to a NiNTA agarose column. The recombinant MOG protein was eluted under non-denaturing conditions using 250 mM Imidazole, dialyzed against 5% propionic acid and H_2O and subsequently lypophilized. The protein concentration was estimated by BCA. The purified MOG protein was visualized on a 12.5% polyacrylamide gel stained with Coomassie blue.

***E. coli* Expression**

The pET.H6 vector containing the truncated human MOG cDNA was introduced into BL21(DE3) cells (Novagen, Madison, WI.) by transformation. Several

colonies were grown together in 2YT medium to an OD of 1.0. The bacteria were then induced overnight with 1mM IPTG. Cells were harvested and lysed with 6M Guanidine/100mM Tris.HCl pH 8.0 at room temperature overnight. The lysate was centrifuged at 20.000 rpm for 30 minutes and the resulting supernatant applied to a NiNTA agarose column (Quiagen, Chatsworth, CA). The protein was eluted with 6M Guanidine/100mM sodium phosphate pH 4.5, dialyzed first against 5% propionic acid, then against H_2O and subsequently lypophilized. The protein concentration was estimated by BCA. The purified MOG protein was visualized on a 12.5% polyacrylamide gel stained with Coomassie blue.

Example 2

Human MOG Expressed in Insect SF-9 to Induce EAE *in vivo*

Two groups of (PLJxSJL)F1 mice were injected with 10 μg and 50 μg respectively N-terminal fragment of recombinant human MOG expressed in insect SF-9 (-TM, recombinant, SF-9) which was prepared according to Examples 1 and 1A herein. The truncated MOG contains amino acids 1-121 (hereinafter intended to refer to MOG 1-121) and was selected, in part, because of its solubility relative to the solubility of amino acids 122-218 of the MOG protein (SEQ ID NO: 2). Human MOG 122-218 (residues 121-218 of SEQ ID NO. 2) is extremely hydrophobic and is believe to include two transmembrane regions. MOG 1-121 was emulsified in complete Freund's adjuvant and injected subcutaneously in mice. At the same time as the MOG 1-121 injection, 200ng pertussis toxin was also injected intravenously. The 200ng pertussis toxin I.V. injection was repeated 2 days later. Emulsions were prepared using MOG 1-121, (-TM, recombinant, SF-9), as described above combined with 400 μg H37Ra (Difco Laboratories, Detroit, MI) in CFA. Beginning at Day 8 after initial immunization, the mice were observed for signs of paralysis and scored daily as an indicator that EAE has been contracted. Mice were scored based on clinical signs according to the following scale: 1, tail paralysis; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, forelimb paralysis; 5, moribund or dead.

The onset of symptoms began as early as 14 days for some mice. The mice were observed for 31 days when the mice were sacrificed, the brains and spinal cords were harvested and histological studies were undertaken to verify the clinical observations. Sixty percent (60%) of mice immunized with 50 μg of rMOG (-TM, recombinant, SF-9) and eighty percent (80%) of mice immunized with 10μg of rMOG (-TM, recombinant, SF-9)exhibited symptoms of EAE.

This procedure was repeated substituting recombinant N-terminal fragment of recombinant human MOG 1-121 expressed in *E.coli* (-TM, recombinant, *E.coli*) for recombinant N-terminal fragment of recombinant human MOG 1-121 expressed in insect SF-9 (-TM, recombinant, SF-9). Preliminary, yet unconfirmed, results indicate similar findings.

Thus, mice induced with EAE in this manner may be useful as an animal model for screening potential therapeutics for treatment of multiple sclerosis. The following is one method for identifying therapeutic compositions for the treatment of multiple sclerosis which comprises the steps of:

administering human MOG to mice in immunogenic form to cause induction of EAE in said mice;

treating said mice induced with EAE with therapeutic compositions comprising at least one antigenic fragment of human MOG prior to the onset of symptoms of EAE or after the onset of symptoms of EAE in said mice; and

determining if said therapeutic composition prevents the onset or progression of the symtoms of EAE in said mice induced with EAE.

Example 3
T-Cell Responses to human MOG Peptide

Peptides were synthesized using either an Applied Biosystems peptide synthesizer or Advanced Chemtech robotics system utilizing FastMOC™ chemistry with commercially available Wang resins, and Fmoc protected amino acids as described previously by Hill et al.(*J.Immunol.* 52: 2890-2898, 1994). The teachings of Hill, Buelow, Gorga and Marshall above cited are incorporated herein by reference.

T-cell assays were performed to further refine the identification of T-cell epitopes. Peripheral blood lymphocytes were isolated from the blood of a human volunteer HLA-DR2 positive donor using Ficoll Hypaque. Twenty (20) million lymphocytes were seeded into 96 wells of a microtiter dish at 2 x 10^5 per well in RPMI culture media supplemented with human AB serum. A mixture of selected MOG peptides was added at a final concentration of 50μM for each peptide. The MOG peptides selected were both naturally occurring peptides and analogs of naturally occurring peptides and were:

MOG 1-13	GQFRVIGPRHPIR (SEQ ID NO: 42)
MOG 20-32	VELPCRISPGKNA (SEQ ID NO: 5)
MOG 70-82, A78	ELLKDAIGAGKVT (analog to MOG 70-82) (SEQ ID NO: 7)

MOG 88-100,K89,S98	VKFSDEGGFTSFF (analog to MOG 88-100) (SEQ ID NO: 9)
MOG 103-115	HSYQEEAAMELKV (SEQ ID NO: 55)
MOG 118-130	PFYWVSPGVLVLL (SEQ ID NO: 56)
MOG 170-182	PHFLRVPCWKITL (SEQ ID NO: 15)

Cultures were incubated in a humidified CO_2 37°C incubator for twelve days, intermittently supplemented with human IL2 (20U/ml) and IL4 (5U/ml). A sample from each culture well was removed, washed to remove previously added peptide, and reseeded into four wells of a fresh microtiter dish (two wells with the peptide mixture and two wells without the peptide mixture for each sample). Autologous irradiated cryopreserved lymphocytes were added as antigen presenting cells. After further 3 days of incubation, 3H-thymidine incorporation was measured. Positive microtiter lines were scored if the mean incorporation in the peptide wells was greater than or equal to 1.5-fold higher than the wells without peptide. Positive microtiter lines were expanded with IL2 and IL4, and then reassayed the following week by the same methods with the individual peptides rather than the peptide mix.

The peptides identified as T-cell epitopes-conatining peptides using this method were MOG 1-13 (SEQ ID NO: 42) and MOG 103-115 (SEQ ID NO: 55). Figure 6 shows the results of the assays performed.

Example 4

Human MS Patient T-cell Response to MOG 20mer peptides and N-terminal Fragment of Recombinant Human MOG Expressed in Insect SF-9 (-TM, recombinant, SF-9)

Peptides (20 amino acids in length) were synthesized using either an Applied Biosystems peptide synthesizer or Advanced Chemtech robotics system utilizing FastMOC™ chemistry with commercially available Wang resins, and Fmoc protected amino acids as described previously by Hill et al.(*J.Immunol.* 52: 2890-2898, 1994). The teachings of Hill, Buelow, Gorga and Marshall above cited are incorporated herein by reference. These peptides are 20 amino acid peptides of human MOG overlapping by 10 amino acids and are shown in Figure 5a.

Following the procedures of Example 3, the T-cell response of a human patient suffering from Multiple Sclerosis was tested with the group of MOG 20mer peptides shown in Figure 5a and N-terminal fragment of recombinant human MOG 1-121 expressed in insect SF-9 (-TM, recombinant, SF-9), which is shown to induce EAE in mice in Example 2. One or more of the MOG peptides tested are suspected of containing at least one T-cell epitope for the autoantigen responsible for Multiple Sclerosis.

The MS patient's PBL were cultured in microtiter wells according to the protocol in Example 3, except that both recombinant MOG 1-121 (48 of 96 wells) and a mixture of 10 MOG fragments (20-mer peptides shown in Figure 5a) (48 of 96 wells) were used separately to initiate the cultures. A sample from each culture well was removed, washed to remove previously added peptide, and reseeded into six wells of a fresh microtiter dish. Six microtiter wells were subsequently found to react with both rMOG and the peptide mixture (2 wells from the cultures initiated with rMOG and 4 wells from the cultures initiated with the peptide mixture). The results confirm that MS patients can have T-cells which can be activated by MOG and which recognize one or more T cell epitopes contained in the MOG peptide mixture. There were also a number of wells that reacted with the peptide mixture but not the recombinant MOG. The significance of these positives is less clear but it is believed that true MOG reactive T cells which recognize an epitope in MOG which is not readily processed *in vitro*.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: DEVAUX, BRIGITTE
ROTHBARD, JONATHAN
SMILEK, DAWN

(ii) TITLE OF INVENTION: USES OF MYELIN OLIGODENDROCYTE GLYCOPROTEIN AND PEPTIDE PORTIONS THEREOF IN PROTOCOLS RELATED TO AUTOIMMUNE DISEASE

(iii) NUMBER OF SEQUENCES: 95

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 610 LINCOLN STREET
(C) CITY: WALTHAM
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02154

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII text

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/116,824
(B) FILING DATE: 03-SEP-1993
(C) CLASSIFICATION:

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(C) REFERENCE/DOCKET NUMBER: 071.1

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1080 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 184..927

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCCGGG GGGCTGTTTC TGCGGGCACA GCTGCAGCAA TTACCGGAGT GGAGGCAGGG
60

CCAGGCAGCA CTGCCTCCAA GATCTTCCCT TGGGCTTTTC AGCAGTAAGG GGACATGCAC
120

CCCAAGGGCC TCCACTTGGC CTGACCTTGC TGCGGGGGCT CTCTGTCCCC AGGAACAGTA
180

GAG ATG GCA AGC TTA TCA AGA CCC TCT CTG CCC AGC TGC CTC TGC TCC
228
Met Ala Ser Leu Ser Arg Pro Ser Leu Pro Ser Cys Leu Cys Ser
1 5 10 15

TTC CTC CTC CTC CTC CTC CTC CAA GTG TCT TCC AGC TAT GCA GGG CAG
276
Phe Leu Leu Leu Leu Leu Leu Gln Val Ser Ser Ser Tyr Ala Gly Gln
20 25 30

TTC AGA GTG ATA GGA CCA AGA CAC CCT ATC CGG GCT CTG GTC GGG GAT
324
Phe Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val Gly Asp
35 40 45

GAA GTG GAA TTG CCA TGT CGC ATA TCT CCT GGG AAG AAC GCT ACA GGC
372
Glu Val Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn Ala Thr Gly
50 55 60

ATG GAG GTG GGG TGG TAC CGC CCC CCC TTC TCT AGG GTG GTT CAT CTC
420
Met Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val Val His Leu
65 70 75

TAC AGA AAT GGC AAG GAC CAA GAT GGA GAC CAG GCA CCT GAA TAT CGG
468
Tyr Arg Asn Gly Lys Asp Gln Asp Gly Asp Gln Ala Pro Glu Tyr Arg
80 85 90 95

GGC CGG ACA GAG CTG CTG AAA GAT GCT ATT GGT GAG GGA AAG GTG ACT
516
Gly Arg Thr Glu Leu Leu Lys Asp Ala Ile Gly Glu Gly Lys Val Thr
100 105 110

CTC AGG ATC CGG AAT GTA AGG TTC TCA GAT GAA GGA GGT TTC ACC TGC
564
Leu Arg Ile Arg Asn Val Arg Phe Ser Asp Glu Gly Gly Phe Thr Cys
115 120 125

TTC TTC CGA GAT CAT TCT TAC CAA GAG GAG GCA GCA ATG GAA TTG AAA
612
Phe Phe Arg Asp His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys
130 135 140

GTA GAA GAT CCT TTC TAC TGG GTG AGC CCT GGA GTG CTG GTT CTC CTC
660
Val Glu Asp Pro Phe Tyr Trp Val Ser Pro Gly Val Leu Val Leu Leu
145 150 155

GCG GTG CTG CCT GTG CTC CTC CTG CAG ATC ACT GTT GGC CTG GTC TTC
708
Ala Val Leu Pro Val Leu Leu Leu Gln Ile Thr Val Gly Leu Val Phe
160 165 170 175

CTC TGC CTG CAG TAC AGA CTG AGA GGA AAA CTT CGA GCA GAG ATA GAG
756

Leu Cys Leu Gln Tyr Arg Leu Arg Gly Lys Leu Arg Ala Glu Ile Glu
180 185 190

AAT CTC CAC CGG ACT TTT GAT CCC CAC TTT CTG AGG GTG CCC TGC TGG
804
Asn Leu His Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro Cys Trp
195 200 205

AAG ATA ACC CTG TTT GTA ATT GTG CCG GTT CTT GGA CCC TTG GTT GCC
852
Lys Ile Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu Val Ala
210 215 220

TTG ATC ATC TGC TAC AAC TGG CTA CAT CGA AGA CTA GCA GGG CAA TTC
900
Leu Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe
225 230 235

CTT GAA GAG CTA CGA AAT CCC TTC TGAGTGATGT CACATCTTGG CAGGGGTGGA
954
Leu Glu Glu Leu Arg Asn Pro Phe
240 245

GGAGAGCCTG GTTGCCCAGG GATTTGTCCT TGGGGACATC TCATCCATCA AGTTGCACAC
1014

TCACTCGCAT CTTTGCTATG GGGACATTCC AATTTGCACT TTCAGGAACA CTCTGAATTC
1074

CAAGTA
1080

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 247 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ser Leu Ser Arg Pro Ser Leu Pro Ser Cys Leu Cys Ser Phe
1 5 10 15

Leu Leu Leu Leu Leu Leu Gln Val Ser Ser Ser Tyr Ala Gly Gln Phe
20 25 30

Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val Gly Asp Glu
35 40 45

Val Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn Ala Thr Gly Met
50 55 60

Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val Val His Leu Tyr
65 70 75 80

Arg Asn Gly Lys Asp Gln Asp Gly Asp Gln Ala Pro Glu Tyr Arg Gly
85 90 95

Arg Thr Glu Leu Leu Lys Asp Ala Ile Gly Glu Gly Lys Val Thr Leu
100 105 110

Arg Ile Arg Asn Val Arg Phe Ser Asp Glu Gly Gi Thr Cys Phe

```
        115                     120                     125
Phe Arg Asp His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys Val
    130                 135                     140

Glu Asp Pro Phe Tyr Trp Val Ser Pro Gly Val Leu Val Leu Leu Ala
145                 150                     155                 160

Val Leu Pro Val Leu Leu Leu Gln Ile Thr Val Gly Leu Val Phe Leu
                165                     170                 175

Cys Leu Gln Tyr Arg Leu Arg Gly Lys Leu Arg Ala Glu Ile Glu Asn
            180                 185                     190

Leu His Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro Cys Trp Lys
        195                     200                 205

Ile Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu Val Ala Leu
    210                 215                     220

Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu
225                 230                     235                 240

Glu Glu Leu Arg Asn Pro Phe
                245
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Gly Gln Phe Arg Val Ile Gly Pro Arg His Pro Ile
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
His Pro Ile Arg Ala Leu Val Gly Asp Glu Val Glu Leu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Val Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val Val His Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Glu Leu Leu Lys Asp Ala Ile Gly Ala Gly Lys Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Lys Phe Ser Asp Glu Gly Gly Phe Thr Ser Phe Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Asp Pro Phe Tyr Trp Val Ser Pro Gly Val Met Val Leu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Pro Phe Tyr Trp Val Ser Pro Gly Val Met Val Leu Met
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Pro Gly Val Leu Val Leu Leu Ala Val Leu Pro Val Leu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Leu Leu Leu Gln Ile Thr Val Gly Leu Val Phe Leu Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Pro His Arg Leu Arg Val Pro Cys Trp Lys Ile Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ile Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Pro Leu Val Ala Leu Ile Ile Cys Tyr Asn Trp Leu His
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val Ala Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Lys Ile Tyr Ser Tyr Phe Pro Ser Val Ile Ser Lys Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ser Arg Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ser Arg Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Asn Val Leu Asp His Leu Thr Cys Arg Ser Ser Gln Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Thr Val Leu Thr Ala Leu Gly Ala Ile Leu Lys Lys Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Thr Leu Leu Gln Ala Ala Pro Ala Leu Asp Lys Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met Arg Val Lys Arg Gly Leu Thr Val Ala Val Ala Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Thr Leu Val Lys Thr Ile Thr Asp Asp Gln Ile Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ala Ala Tyr Ala Ala Ala Lys Ala Ala Ala Ala Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAGAATCCGG GAAGAATGCC ACGGGC
26

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CAGCGGCCGC ACGGAGTTTT CCTCTCAG
28

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CAGAATTCTC AGGTTCTCAG ATGAAGGA
28

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AAGCGGCCGC TATCCTTCAT CTGAGAACCT
30

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CTTTTGAGCA AGTTCAGCCT GGTTAAG
27

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ACCTCGAGGA GGTGGCTTAT GAGTATTTCT TCCAGGGTA
39

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGTGCGGGAA AGGTGACTCT CAGGATCCGG AAT
33

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

ATTCCGGATC CTGAGAGTCA CCTTTCCCGC ACC
33

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Gly Gln Phe Arg Val Ile Gly Pro Arg His Pro Ile Arg
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Phe Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Cys Arg Ile Ser Pro Gly Lys Asn Ala Thr Gly Met Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Thr Gly Met Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val Val His
1 5 10

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Pro Pro Phe Ser Arg Val Val His Leu Tyr Arg Asn Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Arg Val Val His Leu Tyr Arg Asn Gly Lys Asp Gln Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Pro Glu Tyr Arg Gly Arg Thr Glu Leu Leu Lys Asp Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Glu Leu Leu Lys Asp Ala Ile Gly Glu Gly Lys Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Asp Ala Ile Gly Glu Gly Lys Val Thr Leu Arg Ile Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Asp Ala Ile Gly Ala Gly Lys Val Thr Leu Arg Ile Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Gly Phe Thr Cys Phe Phe Arg Asp His Ser Tyr Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Pro Phe Tyr Trp Val Ser Pro Gly Val Leu Val Leu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Leu Val Leu Leu Ala Val Leu Pro Val Leu Leu Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Pro Val Leu Leu Leu Gln Ile Thr Val Gly Leu Val Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Ile Thr Val Gly Leu Val Phe Leu Cys Leu Gln Tyr Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Val Gly Leu Val Phe Leu Cys Leu Gln Tyr Arg Leu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Gly Leu Val Phe Leu Cys Leu Gln Tyr Arg Leu Arg Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Leu Val Phe Leu Cys Leu Gln Tyr Arg Leu Arg Gly Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Leu Cys Leu Gln Tyr Arg Leu Arg Gly Lys Leu Arg Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Leu Gln Tyr Arg Leu Arg Gly Lys Leu Arg Ala Glu Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Gly Lys Leu Arg Ala Glu Ile Glu Asn Leu His Arg Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro Cys Trp
1 5 10

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Pro Leu Val Ala Leu Ile Ile Cys Tyr Asn Trp Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Val Ala Leu Ile Ile Cys Tyr Asn Trp Leu His Arg Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Ala Leu Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Leu Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Gly Gln Phe Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val
1 5 10 15

Gly Asp Glu Val
20

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Pro Ile Arg Ala Leu Val Gly Asp Glu Val Glu Leu Pro Cys Arg Ile
1 5 10 15

Ser Pro Gly Lys
20

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn Ala Thr Gly Met Glu
1 5 10 15

Val Gly Trp Tyr
20

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```
Asn Ala Thr Gly Met Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg
1               5                   10                  15

Val Val His Leu
            20
```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```
Arg Pro Pro Phe Ser Arg Val Val His Leu Tyr Arg Asn Gly Lys Asp
1               5                   10                  15

Gln Asp Gly Asp
            20
```

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

```
Tyr Arg Asn Gly Lys Asp Gln Asp Gly Asp Gln Ala Pro Glu Tyr Arg
1               5                   10                  15

Gly Arg Thr Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

```
Gln Ala Pro Glu Tyr Arg Gly Arg Thr Glu Leu Leu Lys Asp Ala Ile
1               5                   10                  15

Gly Glu Gly Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```
Leu Leu Lys Asp Ala Ile Gly Glu Gly Lys Val Thr Leu Arg Ile Arg
1               5                   10                  15

Asn Val Arg Phe
            20
```

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

```
Val Thr Leu Arg Ile Arg Asn Val Arg Phe Ser Asp Glu Gly Gly Phe
1               5                   10                  15

Thr Cys Phe Phe
            20
```

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

```
Ser Asp Glu Gly Gly Phe Thr Cys Phe Phe Arg Asp His Ser Tyr Gln
1               5                   10                  15

Glu Glu Ala Ala
            20
```

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```
Arg Asp His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys Val Glu
1               5                   10                  15

Asp Pro Phe Tyr
            20
```

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```
Met Glu Leu Lys Val Glu Asp Pro Phe Tyr Trp Val Ser Pro Gly Val
1               5                   10                  15

Leu Val Leu Leu
            20
```

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Trp Val Ser Pro Gly Val Leu Val Leu Leu Ala Val Leu Pro Val Leu
1 5 10 15

Leu Leu Gln Ile
20

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Ala Val Leu Pro Val Leu Leu Leu Gln Ile Thr Val Gly Leu Val Phe
1 5 10 15

Leu Cys Leu Gln
20

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Thr Val Gly Leu Val Phe Leu Cys Leu Gln Tyr Arg Leu Arg Gly Lys
1 5 10 15

Leu Arg Ala Glu
20

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Tyr Arg Leu Arg Gly Lys Leu Arg Ala Glu Ile Glu Asn Leu His Arg

```
1               5                   10                  15
Thr Phe Asp Pro
            20
```

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

```
Ile Glu Asn Leu His Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro
1               5                   10                  15
Cys Trp Lys Ile
            20
```

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

```
His Phe Leu Arg Val Pro Cys Trp Lys Ile Thr Leu Phe Val Ile Val
1               5                   10                  15
Pro Val Leu Gly
            20
```

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

```
Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu Val Ala Leu Ile
1               5                   10                  15
Ile Cys Tyr Asn
```

20

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

```
Pro Leu Val Ala Leu Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu
1               5                   10                  15

Ala Gly Gln Phe
            20
```

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```
Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu Glu Glu Leu Arg Asn
1               5                   10                  15

Pro Phe
```

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

```
AGCTCGAGCC GCGGAGGGCA GTTCAGAGTG ATA
33
```

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GACTCGAGTC ACCAGTAGAA AGGATCTTC
29

Claims

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding human myelin oligodendrocyte glycoprotein (MOG) or at least one antigenic fragment thereof.

2. The nucleic acid molecule of claim 1 wherein said nucleotide sequence consists essentially of nucleotides encoding amino acids 1-218 of Fig. 1 (SEQ ID NO: 2) or an allelic variant thereof:

3. An oligomer comprising a nucleotide sequence based on the nucleotide sequence of Fig. 1 (SEQ ID NO: 1).

4. An expression system which is a DNA molecule comprising a nucleotide sequence encoding human MOG or an antigenic fragment thereof, operably linked to control sequences for expression in a compatible host.

5. The expression system of claim 4 wherein said nucleotide sequence encodes amino acids 1-218 of Figure 1 (SEQ ID NO: 2) or an antigenic fragment portion thereof.

6. A recombinant host cell modified to contain the expression system of claim 4.

7. Isolated and purified human MOG or an antigenic fragment thereof.

8. A method to produce human MOG, or an antigenic fragment thereof, comprising the steps of:

a) culturing a host cell modified to contain an expression system directing expression of a nucleotide sequence encoding human MOG or fragment thereof in an appropriate medium, to produce a culture containing human MOG or fragment thereof; and

b) recovering said human MOG or fragment thereof from the culture.

9. A peptide comprising at least one T-cell epitope of human MOG or tandem copies thereof.

10. The peptide of claim 9 wherein said peptide comprises any of amino acid sequences as shown in Figure 2 (SEQ ID NOS: 4-9, 11, 15, 16, and 42-72), Figure 5a and 5b.

11. A peptide comprising at least two T-cell epitopes of human MOG.

12. A modified form of human MOG, or antigenic fragment thereof wherein said modification comprises substituting for one or more amino acids of said peptide or protein a different amino acid which does not interfere with the biological activity of said peptide or protein.

13. A nucleic acid molecule comprising a nucleotide sequence encoding the peptide of any one of claims 9-12.

14. An expression system which is a DNA molecule comprising a nucleotide sequence encoding the peptide of any one of claims 9-12 operably linked to control sequences for expression in a compatible host.

15. A recombinant host cell modified to contain the expression system of claim 14.

16. A method to produce the peptide of any one of claims 9-12 which comprises:

a) culturing a host cell modified to contain an expression system directing expression of a nucleotide sequence encoding said peptide in an appropriate medium to produce a culture containing said peptide; and

b) recovering the peptide from the culture.

17. A modified form of human MOG or an antigenic fragment thereof or of a peptide of any of claims 9-12 wherein said modification comprises coupling said protein, fragment or peptide to an additional moiety selected from the group consisting of polyethyleneglycol, a moiety that enhances the solubility of the protein, fragment or

peptide, a moiety which facilitates purification of said protein, fragment or peptide and a moiety which comprises a proteolytic cleavage site.

18. A therapeutic composition comprising isolated human MOG and/or at least one antigenic fragment thereof and/or at least one peptide of any of claims 9-12 or 16 and a pharmaceutically acceptable carrier or diluent.

19. The therapeutic composition of claim 11 wherein said human MOG comprises the amino acid sequence of positions 1-218 shown in Figure 1 (SEQ ID NO: 2) or an amino acid sequence encoded by an allelic variant of the nucleotide sequence of Figure 1 (SEQ ID NO: 1) or an antigenic fragment thereof.

20. A method of treating a demyelinating autoimmune disease comprising administering to a subject a therapeutically effective amount of the composition of claim 19.

21. A method of diagnosing a demyelinating autoimmune disease comprising combining a blood sample obtained from a subject to be tested with isolated human MOG, or antigenic fragment thereof or a peptide of any of claims 9-12 and 16 under conditions appropriate for binding of blood components with MOG or fragment thereof, and determining the extent to which such binding occurs.

22. The method of claim 21 wherein the extent to which binding occurs is determined by assessing T-cell function, T-cell proliferation, B-cell function, binding of the protein or fragment thereof to antibodies present in the blood or a combination thereof.

23. A monoclonal antibody or immunoreactive fragment thereof, specifically reactive with human MOG or antigenic fragment thereof or a peptide of any of claims 9-12 and 16.

24. A method to identify a T-cell epitope of human MOG which method comprises

identifying a hydrophobic amino acid contained in human MOG;

determining the nature of the amino acid in the human MOG sequence separated N__C from said hydrophobic amino acid by an intervening four amino acids;

determining whether said amino acid is a small amino acid selected from the group consisting of glycine, alanine, serine, threonine, and cysteine or is a different amino acid; and

identifying as a T-cell epitope a peptide which contains said hydrophobic and small amino acid wherein said small amino acid is separated by 4 amino acids N__C from said hydrophobic amino acid.

25. An isolated peptide of human MOG said peptide comprising at least one T-cell epitope, said peptide comprising a sequence of amino acid residues selected from the group consisting of MOG 1-13 (SEQ ID NO. 42), MOG 103-115 (SEQ. ID NO. 55), MOG 1-121 (first 121 amino acids of SEQ ID NO. 2), MOG 1-20 (SEQ ID NO. 73), MOG 11-30 (SEQ ID NO. 74), MOG 21-40 (SEQ. ID NO.75), MOG 31-50 (SEQ ID NO.76), MOG 41-60 (SEQ ID NO.77), MOG 51-70 (SEQ ID NO 78), MOG 61-80 (SEQ ID NO 79), MOG 71-90 (SEQ ID NO.80), MOG 91-110 (SEQ ID NO. 82), and MOG 101-120 (SEQ ID NO. 83).

26. A mixture of peptides human MOG , said mixutre comprising MOG 1-20 (SEQ ID NO. 73), MOG 11-30 (SEQ ID NO. 74), MOG 21-40 (SEQ. ID NO.75), MOG 31-50 (SEQ ID NO.76), MOG 41-60 (SEQ ID NO.77), MOG 51-70 (SEQ ID NO 78), MOG 61-80 (SEQ ID NO 79), MOG 71-90 (SEQ ID NO.80), MOG 91-110 (SEQ ID NO. 82), and MOG 101-120 (SEQ ID NO. 83) wherein said mixture comprises at least one T-cell epitope.

27. A method for identifying therapeutic compositions for the treatment of multiple sclerosis comprising the steps of:

administering human MOG to mice in immunogenic form to cause induction of EAE in said mice;

treating said mice induced with EAE with at least one therapeutic composition comprising at least one antigenic fragment of human MOG prior to the onset of symptoms of EAE or after the onset of symptoms of EAE in said mice; and

determining if said at least one therapeutic composition prevents the onset or progression of the symtoms of EAE in said mice induced with EAE.

```
1                                       31
GAA TTC CGG GGG GCT GTT TCT GCG GGC ACA GCT GCA GCA ATT ACC GGA GTG GAG GCA GGG

61                                      91
CCA GGC AGC ACT GCC TCC AAG ATC TTC CCT TGG GCT TTT CAG CAG TAA GGG GAC ATG CAC

121                                     151
CCC AAG GGC CTC CAC TTG GCC TGA CCT TGC TGC GGG GGC TCT CTG TCC CCA GGA ACA GTA

181                                     211
GAG ATG GCA AGC TTA TCA AGA CCC TCT CTG CCC AGC TGC CTC TGC TCC TTC CTC CTC CTC
    met ala ser leu ser arg pro ser leu pro ser cys leu cys ser phe leu leu leu
241                                     271                                  10
CTC CTC CTC CAA GTG TCT TCC AGC TAT GCA GGG CAG TTC AGA GTG ATA GGA CCA AGA CAC
leu leu leu gln val ser ser ser tyr ala gly gln phe arg val ile gly pro arg his
301                                     331                                  30
CCT ATC CGG GCT CTG GTC GGG GAT GAA GTG GAA TTG CCA TGT CGC ATA TCT CCT GGG AAG
pro ile arg ala leu val gly asp glu val glu leu pro cys arg ile ser pro gly lys
361                                     391                                  50
AAC GCT ACA GGC ATG GAG GTG GGG TGG TAC CGC CCC CCC TTC TCT AGG GTG GTT CAT CTC
asn ala thr gly met glu val gly trp tyr arg pro pro phe ser arg val val his leu
421                                     451                                  70
TAC AGA AAT GGC AAG GAC CAA GAT GGA GAC CAG GCA CCT GAA TAT CGG GGC CGG ACA GAG
tyr arg asn gly lys asp gln asp gly asp gln ala pro glu tyr arg gly arg thr glu
481                                     511                                  90
CTG CTG AAA GAT GCT ATT GGT GAG GGA AAG GTG ACT CTC AGG ATC CGG AAT GTA AGG TTC
leu leu lys asp ala ile gly glu gly lys val thr leu arg ile arg asn val arg phe
```

Fig. 1

```
541                                     571                                     110
TCA GAT GAA GGA GGT TTC ACC TGC TTC TTC CGA GAT CAT TCT TAC CAA GAG GAG GCA GCA
ser asp glu gly gly phe thr cys phe phe arg asp his ser tyr gln glu glu ala ala
601                                     631                                     130
ATG GAA TTG AAA GTA GAA GAT CCT TTC TAC TGG GTG AGC CCT GGA GTG CTG GTT CTC CTC
met glu leu lys val glu asp pro phe tyr trp val ser pro gly val leu val leu leu
661                                     691                                     150
GCG GTG CTG CCT GTG CTC CTC CTG CAG ATC ACT GTT GGC CTC GTC TTC CTC TGC CTG CAG
ala val leu pro val leu leu leu gln ile thr val gly leu val phe leu cys leu gln
721                                     751                                     170
TAC AGA CTG AGA GGA AAA CTT CGA GCA GAG ATA GAG AAT CTC CAC CGG ACT TTT GAT CCC
tyr arg leu arg gly lys leu arg ala glu ile glu asn leu his arg thr phe asp pro
781                                     811                                     190
CAC TTT CTG AGG GTG CCC TGC TGG AAG ATA ACC CTG TTT GTA ATT GTG CCG GTT CTT GGA
his phe leu arg val pro cys trp lys ile thr leu phe val ile val pro val leu gly
841                                     871                                     210
CCC TTG GTT GCC TTG ATC ATC TGC TAC AAC TGG CTA CAT CGA AGA CTA GCA GGG CAA TTC
pro leu val ala leu ile ile cys tyr asn trp leu his arg arg leu ala gly gln phe
901                         218         931
CTT GAA GAG CTA CGA AAT CCC TTC TGA GTG ATG TCA CAT CTT GGC AGG GGT GGA GGA GAG
leu glu glu leu arg asn pro phe OPA
                                        991
    GGT TGC CCA GGG ATT TGT CCT TGG GGA CAT CTC ATC CAT CAA GTT GCA CAC TCA CTG

1021                                    1051
GCA TCT TTG CTA TGG GGA CAT TCC AAT TTG CAC TTT CAG GAA CAC TCT GAA TTC CAA GTA
```

Fig. 1 cont.

PEPTIDE	SEQUENCE	DRB1*1501 IC50 (nM)	DRB5*0101 IC50 (nM)
HUMAN MOG 1-13	H:-GQFRVIGPRHPIR-NH2	93	17
HUMAN MOG 3-15	H:-FRVIGPRHPIRAL-NH2	5857	198
HUMAN MOG 4-16	H:-RVIGPRHPIRALV-NH2	32656	8037
HUMAN MOG 10-22	H:-HPIRALVGDEVEL-NH2	100000	100000
HUMAN MOG 20-32	H:-VELPCRISPGKNA-NH2	6355	7635
HUMAN MOG 24-36	H:-CRISPGKNATGME-NH2	1432	69428
HUMAN MOG 33-45	H:-TGMEVGWYRPPFS-NH2	47500	100000
HUMAN MOG 35-47	H:-MEVGWYRPPFSRV-NH2	2257	135
HUMAN MOG 37-49	H:-VGWYRPPFSRVVH-NH2	7705	479
HUMAN MOG 38-50	H:-GWYRPPFSRVVHL-NH2	118	246
HUMAN MOG 42-54	H:-PPFSRVVHLYRNG-NH2	13124	17
HUMAN MOG 46-58	H:-RVVHLYRNGKDQD-NH2	100000	59017
HUMAN MOG 63-75	H:-PEYRGRTELLKDA-NH2	5411	66
HUMAN MOG 70-82	H:-ELLKDAIGEGKVT-NH2	2890	1970
HUMAN MOG 70-82,A78	H:-ELLKDAIGAGKVT-NH2	587	501
HUMAN MOG 74-86	H:-DAIGEGKVTLRIR-NH2	31000	975
HUMAN MOG 74-86,A78	H:-DAIGAGKVTLRIR-NH2	5008	247
HUMAN MOG 88-100,K89,S98	H:-VKFSDEGGFTSFF-NH2	137	185
HUMAN MOG 94-106	H:-GGFTCFFRDHSYQ-NH2	11150	7389
HUMAN MOG 103-115	H:-HSYQEEAAMELKV-NH2	1201	697

Fig. 2

PEPTIDE	SEQUENCE	DRB1*1501 IC50 (nM)	DRB5*0101 IC50 (nM)
HUMAN MOG 117-129	H:-DPFYWVSPGVLVL-NH2	12840	83
HUMAN MOG 118-130	H:-PFYWVSPGVLVLL-NH2	534	238
HUMAN MOG 127-139	H:-LVLLAVLPVLLLQ-NH2	1708	467
HUMAN MOG 134-146	H:-PVLLLQITVGLVF-NH2	17725	8753
HUMAN MOG 140-152	H:-ITVGLVFLCLQYR-NH2	1260	786
HUMAN MOG 142-154	H:-VGLVFLCLQYRLR-NH2	1735	75
HUMAN MOG 143-155	H:-GLVFLCLQYRLRG-NH2	907	240
HUMAN MOG 144-156	H:-LVFLCLQYRLRGK-NH2	747	119
HUMAN MOG 147-159	H:-LCLQYRLRGKLRA-NH2	7677	50
HUMAN MOG 149-161	H:-LQYRLRGKLRAEI-NH2	1443	139
HUMAN MOG 155-167	H:-GKLRAEIENLHRT-NH2	2803	178
HUMAN MOG 166-178	H:-RTFDPHFLRVPCW-NH2	1844	43
HUMAN MOG 170-182	H:-PHFLRVPCWKITL-NH2	100000	100000
HUMAN MOG 182-194	H:-ITLFVIVPVLGPL-NH2	1029	100000
HUMAN MOG 192-204	H:-GPLVALIICYNWL-NH2	693	498
HUMAN MOG 195-207	H:-VALIICYNWLHRR-NH2	16	5
HUMAN MOG 196-208	H:-ALIICYNWLHRRL-NH2	404	119
HUMAN MOG 197-209	H:-LIICYNWLHRRLA-NH2	1280	461
HUMAN MOG 199-211	H:-ICYNWLHRRLAGQ-NH2	1571	3806
HUMAN MOG 201-213	H:-YNWLHRRLAGQFL-NH2	100000	100000

Fig. 2 cont.

AMINO ACID	2	3	4	5	6	7	8	9	10	11	12
A	1.0	235.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C	2.2	6818.2	1.9	0.3	0.4	1.0	0.8	0.7	0.8	0.9	0.7
D	3.9	6818.2	7.0	2.4	0.9	1.4	7.8	4.3	2.2	26.0	1.6
E	1.4	6818.2	12.0	3.8	0.5	1.0	7.0	5.0	1.4	31.0	2.2
F	0.7	1.7	0.4	0.6	0.3	0.8	12.4	1.9	0.6	36.0	0.4
G	0.7	1570.5	4.2	0.8	2.0	1.0	1.1	2.0	1.5	1.4	1.1
H	2.4	6818.2	0.9	2.1	0.7	0.9	2.9	0.5	2.2	4.0	1.1
I	2.0	6.4	0.4	0.9	0.8	0.7	0.8	1.2	0.7	11.7	1.6
K	1.2	6818.2	0.4	2.7	30.0	1.3	13.3	20.0	1.1	23.0	1.1
L	1.5	15.4	0.4	0.5	0.6	1.8	1.4	1.0	0.9	4.2	0.8
M	1.2	8.3	1.4	0.5	0.3	0.7	3.3	0.8	0.8	4.5	0.7
N	2.0	4159.1	0.7	2.6	0.2	1.2	0.6	1.2	0.9	5.0	1.4
P	7.6	83.6	544.0	1.7	70.8	3.0	1.5	0.8	0.7	5.4	1.2
Q	0.7	6818.2	0.9	4.2	0.6	1.6	1.9	8.5	0.6	5.4	0.6
R	1.5	6818.2	0.4	2.7	18.3	1.1	17.2	14.0	1.3	18.0	1.4
S	1.5	6818.2	0.7	0.4	0.8	1.0	0.7	1.6	2.6	1.7	1.2
T	0.7	371.6	0.8	1.3	1.2	0.7	0.6	1.7	3.0	6.9	0.8
V	1.1	14.1	0.5	1.0	1.0	0.8	0.9	1.2	0.6	6.2	1.8
W	3.2	1.2	0.6	1.8	1.5	0.7	2.8	1.5	1.2	7.8	0.6
Y	1.2	1.0	0.6	0.7	0.6	0.5	3.2	0.4	0.4	10.2	0.4

Fig. 3

PEPTIDE	SEQUENCE													PREDICTED IC50 (nM)	EXPERIMENTAL IC50 (nM)
TETANUS 591-603	K	I	Y	S	Y	F	P	S	V	I	S	K	V	12	9.5
COMPONENT RATIO		2.0	1.0	0.7	0.7	0.3	3.0	0.7	1.2	0.7	1.7	1.1			
HA 103-115	P	D	Y	A	S	L	R	S	L	V	A	S	S	9	19
COMPONENT RATIO		3.9	1.0	1.0	0.4	0.6	1.1	0.7	1.0	0.6	1.0	1.2			
RMBP 90-102	H	F	F	K	N	I	V	T	P	R	T	P	P	46	40
COMPONENT RATIO		0.7	1.7	0.4	2.6	0.8	0.8	0.6	0.8	1.3	6.9	1.2			
HA 307-319	P	K	Y	V	K	Q	N	T	L	K	L	A	T	44	40
COMPONENT RATIO		1.2	1.0	0.5	2.7	0.6	1.2	0.6	1.0	1.1	4.2	1.0			
FLU NP 383-395	S	R	Y	W	A	I	R	T	R	S	G	G	I	356	140
COMPONENT RATIO		1.5	1.0	0.6	1.0	0.8	1.1	0.6	14.0	2.6	1.4	1.1			
TETANUS 828-840	M	Q	Y	I	K	A	N	S	K	F	I	G	I	1272	2800
COMPONENT RATIO		0.7	1.0	0.4	2.7	1.0	1.2	0.7	20.0	0.6	11.7	1.1			
MYOGLOBIN 67-79	T	V	L	T	A	L	G	A	I	L	K	K	K	3299	4800
COMPONENT RATIO		1.1	15.4	0.8	1.0	0.6	1.0	1.0	1.2	0.9	23.0	1.1			
HA 23-35	G	T	L	V	K	T	I	T	D	D	Q	I	E	7431	6700
COMPONENT RATIO		0.7	15.4	0.5	2.7	1.2	0.7	0.6	4.3	2.2	5.4	1.6			
TUB 19KD 2-14	H	R	V	K	R	G	L	T	V	A	V	A	G	4445	13000
COMPONENT RATIO		1.5	14.1	0.4	2.7	2.0	1.8	0.6	1.2	1.0	6.2	1.0			
TUB 65KD 416-428	T	L	L	Q	A	A	P	A	L	D	K	L	K	33321	52000
COMPONENT RATIO		1.5	15.4	0.9	1.0	1.0	3.0	1.0	1.0	2.2	23.0	0.8			
PERTUSSIS 31-43	N	V	L	D	H	L	T	G	R	S	S	Q	V	55314	95000
COMPONENT RATIO		1.1	15.4	7.0	2.1	0.6	0.7	1.1	14.0	2.6	1.7	0.6			
MATRIX 18-30	G	P	L	K	A	E	I	A	Q	R	L	E	D	24062	100000
COMPONENT RATIO		7.6	15.4	0.4	1.0	0.5	0.7	1.0	8.5	1.3	4.2	2.2			

Fig. 4

PEPTIDE	SEQUENCE	# AA
1-20	G Q F R V I G P R H P I R A L V G D E V	20
11-30	P I R A L V G D E V E L P C R I S P G K	20
21-40	E L P C R I S P G K N A T G M E V G W Y	20
31-50	N A T G M E V G W Y R P P F S R V V H L	20
41-60	R P P F S R V V H L Y R N G K D Q D G D	20
51-70	Y R N G K D Q D G D Q A P E Y R G R T E	20
61-80	Q A P E Y R G R T E L L K D A I G E G K	20
71-90	L L K D A I G E G K V T L R I R N V R F	20
91-110	S D E G G F T C F F R D H S Y Q E E A A	20
101-120	R D H S Y Q E E A A M E L K V E D P F Y	20

Fig. 5A

MOG 1-121

G Q F R V I G P R H P I R A L V G D E V
E L P C R T S P G K N A T G M E V G W Y
R P P F S R V V H L Y R N G K D Q D G D
Q A P E Y R G R T E L L K D A I G E G K
V T L R I R N V R F S D E G G F T C F F
R D H S Y Q E E A A M E L K V E D P F Y W

Fig. 5B

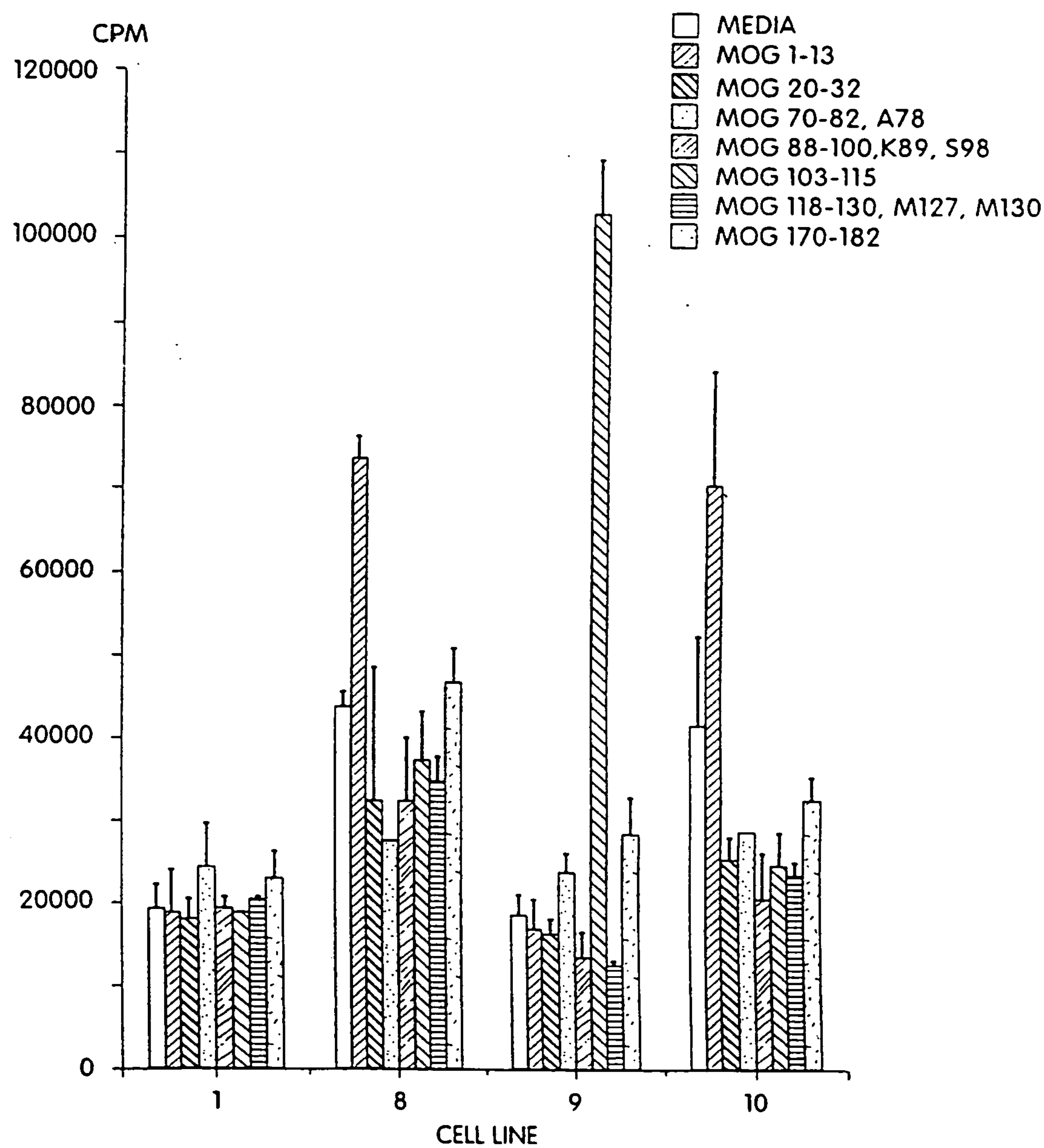

Fig. 6

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